PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Abdelhamid ELAISSARI, David DURACHER, Christian PICHOT, Francois MALLET and Armelle NOVELLI-ROUSSEAU

Attn: PCT Branch

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For: PROCESS FOR ISOLATING A TARGET BIOLOGICAL MATERIAL, CAPTURE PHASE, DETECTION PHASE AND REAGENT

TRANSLATION OF THE ANNEXES TO THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Attached hereto is a translation of the annexes to the International Preliminary Examination Report (Form PCT/IPEA/409). The attached translated material replaces pages 1-3 and the claims.

Respectfully submitted,

Reg No. 40,085

William P. Berridge
Registration No. 30,024

Thomas J. Pardini
Registration No. 30,411

WPB:TJP/sfe

OLIFF & BERRIDGE, PLC
P.O. Box 19928
Alexandria, Virginia 22320
Telephone: (703) 836-6400

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VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below;

That I am knowledgeable in the French language in which the below identified international application was filed, and that, to the best of my knowledge and belief, the English translation of the amended sheets of the international application No. PCT/FR98/00772 is a true and complete translation of the amended sheets of the above identified international application as filed.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application issued thereon.

Date: 22 September 1999

Full name of the translator : Norval O'CONNOR
For and on behalf of RWS Group plc

Post Office Address : Europa House, Marsham Way,
Gerrards Cross, Buckinghamshire,
England.

Process for isolating a target biological material, capture phase, detection phase and reagent

The present invention relates to the isolation or detection of a biological material, referred to as the target biological material, contained in a sample, by means of a process using a capture phase, and optionally a detection phase, according to which said material is exposed to the capture phase at least, and the capture phase/target biological material complex formed is then detected, optionally with said detection phase.

In the presentation of the invention which follows, reference is made in particular to the isolation of a target protein biological material, but, needless to say, the scope of the invention should not be limited thereto.

Thus, according to the invention, the expression "biological material" means, in particular, a protein or glycoprotein material such as an antigen, a hapten, an antibody, a protein, a peptide, an enzyme or a substrate, and fragments thereof; but also a nucleic material such as a nucleic acid (DNA or RNA), a nucleic acid fragment, a probe or a primer; a hormone.

According to EP-A-0,516,198, a process is described for detecting a biological material using a capture phase consisting of magnetic particles. This capture phase is obtained by co-precipitation of a transition metal and of a polymer which have available coordination sites to which the atoms of said metal come to bind, followed by binding of a biological species to reactive sites of said polymer via a bifunctional agent, said species having an affinity for the material to be detected.

In accordance with the article by M. Kempe et al. (1), a process is known for capturing a target protein which contains polyhistidine sequences, namely RNase A, according to which the high affinity of the imidazole group of histidine for metals is used.

This process comprises the following steps:

- a capture phase is used consisting of silica particles functionalized with methacrylate groups,

- a target protein and a metal-complexing agent, namely N-(4-vinyl)benzyliminodiacetic acid (VBIDA), are placed in contact with a metal, in order to obtain a complex resulting from coordination bonding between the metal and the imidazole groups of the histidine, and coordination bonding between the metal and the carboxyl groups of VBIDA, and

- said functionalized silica particles are placed in contact with the complex formed above.

This immobilization process does not lead to optimum binding of the target protein.

Document US-A-4,246,350 described a process for immobilizing an enzyme using a capture phase which consists of a macroporous polymer containing complexing groups linked to a transition metal. The drawback of such a capture phase results directly from the macroporous nature of the polymer. The reason for this is that, although this macroporous nature makes it possible to maximize the adsorption of the enzyme onto the capture phase, it becomes disadvantageous at the time of isolation of the enzyme using a detection phase, since the proportion of enzyme adsorbed in the polymer pores will not be accessible to said detection phase.

According to the present invention, a process is provided for isolating a target biological material, using a capture phase such that it makes it possible to optimize the binding of this material on this phase, while at the same time reducing, or even eliminating, any side reaction of adsorption of said material onto said capture phase. The interaction between the capture phase and the biological material is specific, thus making it possible, during isolation, to detect the proportion of biological material effectively bound to the capture phase.

For this purpose, the process for isolating a target biological material uses a capture phase which has the following properties:

it is in microparticulate form or in linear form, it consists of at least one first particulate or linear polymer, of hydrophilic apparent nature, and first complexing groups, linked covalently,

the first complexing groups are linked by coordination to a first transition metal,

the first transition metal is itself linked by chelation to a first biological species which is capable of specifically recognizing the target biological material.

According to one variant of the process of the invention, the capture phase defined above comprises a marker, in order to obtain a detection phase.

According to another variant of the process, a detection phase is also used which has the following properties:

it in microparticulate or linear form,

it consists of at least one second particulate or linear polymer, of hydrophilic apparent nature, and second complexing groups,

the second complexing groups are linked by coordination to a second transition metal,

the second transition metal is itself linked by chelation to a second biological species capable of specifically recognizing the target biological material,

it comprises a marker.

According to the invention, the term "microparticulate" means in the form of particles not more than 10 Πm in size. Preferably, they do not exceed 5 Πm in size.

The first and/or second particulate or linear polymer is advantageously a hydrophilic polymer, and in particular a functionalized polymer obtained by

CLAIMS

1. Process for isolating a target biological material contained in a sample, according to which a capture phase is provided, said target biological material is placed in contact with at least the capture material, and the capture phase/target biological material complex is detected,

said process being characterized in that,

the capture phase is in microparticulate or linear form and consists of at least one first particulate or linear polymer, with a hydrophilic apparent nature and first complexing groups, these groups being linked covalently to said first polymer and by coordination by a first transition metal, which is itself linked by chelation to a first biological species capable of specifically recognizing the target biological material.

2. Process according to Claim 1, characterized in that the capture phase comprises a marker in order to obtain a phase for detecting said biological material.

3. Process according to Claim 1, characterized in that a detection phase is also provided, which is in microparticulate or linear form and consists of at least one second particulate or linear polymer, of hydrophilic apparent nature, and second complexing groups, these groups being linked by coordination to a second transition metal, which is itself linked to a second biological species capable of specifically recognizing the target biological material, and a marker.

4. Process according to Claim 1, characterized in that the first polymer is chosen from the group of hydrophilic polymers.

5. Process according to Claim 3 or according to the combination of Claims 4 and 3, characterized in that the second polymer is chosen from the group of hydrophilic polymers.

6. Process according to Claim 4, characterized in that the first polymer is a functionalized polymer obtained by polymerization of a water-soluble monomer, of acrylamide, of an acrylamide derivative, of methacrylamide or of a methacrylamide derivative, of at least one crosslinking agent and of at least one functional monomer.

7. Process according to Claim 5, and optionally Claim 6, characterized in that the second polymer is a functionalized polymer obtained by polymerization of a water-soluble monomer, of acrylamide, of an acrylamide derivative, of methacrylamide or of a methacrylamide derivative, of at least one crosslinking agent and of at least one functional monomer.

8. Process according to Claim 6 and/or 7, characterized in that the water-soluble monomer is chosen from N-isopropylacrylamide, N-ethylmethacrylamide, N-n-propylacrylamide, N-n-propylmethacrylamide, N-n-isopropylmethacrylamide, N-cyclopropylacrylamide, N,N-diethylacrylamide, N-methyl-N-isopropylacrylamide and N-methyl-N-n-propylacrylamide, the monomer preferably being N-isopropylacrylamide (NIPAM).

9. Process according to Claim 6 and/or 7, characterized in that the functional monomer corresponds to formula I below:

$$CH_2=C(Z)-(X)_m-(R)_p-(Y)_n \quad (I)$$

Z represents H, a C1-C5 alkyl radical or a benzyl, -COOH or $-CO-NH-CH(CH_3)_2$ radical,

Y represents $-CH_2-COOH$, $-N(CH_2-COOH)_2$, $-N(CH-COOH)(CH_2-COOH)$, or $-N(CH_2-CH_2-NH_2)_2$

$$|$$
$$(CH_2-COOH)$$

X represents $-NH(CH_2-CH_2-)$, $--N(CH_2-CH_2-)_2$, $-N(CH_2-COOH)(CH_2-CH_2-)$, or $CH(COOH)-$,

R represents a linear hydrocarbon-based chain, optionally interrupted with at least one hetero atom such as O or N,

m and p are each an integer which, independently of each other, are equal to 0 or 1, and

n is an integer ranging between 1 and 3.

10. Process according to Claim 9, characterized in that the functional monomer is chosen from carboxylic derivatives, optionally containing nitrogen, itaconic acid, acrylic derivatives and methacrylic derivatives.

11. Process according to any one of Claims 1 to 10, characterized in that the capture phase is in microparticulate form and in that the average particle size is not more than 5 µm.

12. Process according to any one of Claims 3 and 5 to 11, characterized in that the detection phase is in microparticulate form and in that the average particle size is not more than 5 µm.

13. Process according to Claim 1, characterized in that the capture phase also comprises a flat or particulate support.

14. Process according to Claim 13, characterized in that the support is particulate and consists of an organic or inorganic, hydrophilic or hydrophobic core.

15. Process according to Claim 14, characterized in that said core is chosen from the group comprising polystyrene, silica and metal oxides.

16. Process according to Claim 14 or 15, characterized in that said core also contains a magnetic compound.

17. Process according to any one of Claims 14 to 16, characterized in that said core is coated with said first polymer, the latter being linear.

18. Process according to any one of Claims 14 to 16, characterized in that said core is coated with said first polymer, said polymer being particulate.

19. Process according to Claim 1, characterized in that the first polymer is poly(N-isopropylacrylamide) and the complexing groups are derived from itaconic acid or from maleic anhydride-co-methyl vinyl ether.

20. Process according to Claim 3 or according to the combination of Claims 3 and 19, characterized in that the second polymer is poly(N-isopropylacrylamide) and the complexing groups are derived from itaconic acid or from maleic anhydride-co-methyl vinyl ether.

21. Process according to Claim 1, characterized in that the first transition metal is chosen from zinc, nickel, copper, cobalt, iron, magnesium, manganese, lead, palladium, platinum and gold.

22. Process according to Claim 3 or according to the combination of Claims 3 and 21, characterized in that the second transition metal is chosen from zinc, nickel, copper, cobalt, iron, magnesium, manganese, lead, palladium, platinum and gold.

23. Process according to Claim 1, characterized in that the placing in contact of the first biological species with the capture phase is carried out at a pH above or equal to the isoelectric point of said first biological species.

24. Process according to Claim 3 or according to the combination of Claims 3 and 23, characterized in that the placing in contact of the second biological species with the detection phase is carried out at a pH above or equal to the isoelectric point of said second biological species.

25. Process according to Claim 1, characterized in that the first biological species is rich in histidine and/or cysteine.

26. Process according to Claim 3 or according to the combination of Claims 3 and 25, characterized in that the second biological species is rich in histidine and/or in cysteine.

27. Process according to Claim 1, characterized in that an agglutination reaction is used.

28. Process according to Claim 2 or 3, characterized in that the marker for the detection phase is chosen from the group consisting of an enzyme, biotin, iminobiotin, a fluorescent component, a

radioactive component, a chemiluminescent component, an electron-density component, a magnetic component, an antigen, a hapten and an antibody.

29. Process according to Claim 2 or 3, characterized in that the ELISA technique is used.

30. Use of a first particulate or linear polymer, of hydrophilic apparent nature, and first complexing groups, these groups being linked by coordination to a first transition metal, which is itself linked to a first biological species capable of recognizing the target biological material, as a phase for capturing a target biological material, in microparticulate or linear form.

31. Use of a second particulate or linear polymer, of hydrophilic apparent nature, and second complexing groups, these groups being linked by coordination to a second transition metal, which is itself linked to a second biological species capable of recognizing the target biological material, and a marker, as a phase for detecting a target biological material, in microparticulate or linear form.

32. Use of a first polymer according to Claim 30 and/or use of a second polymer according to Claim 31, in a reagent for isolating a target biological material.